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(73) Proprietor: **MEASUREX CORPORATION**
One Results Way
Cupertino, California 95014(US)

(72) Inventor: **Alguard, Mark J.**
1020 Waverley Street
Palo Alto California 94301(US)

(74) Representative: **Atkinson, Peter Birch et al**
Marks & Clerk Suite 301 Sunlight House
Quay Street
Manchester M3 3JY(GB)

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Description

The present invention relates to color sensors, and more particularly, to sensors for measuring the reflective and fluorescent properties of various objects such as paper.

5 In industry, it is often important to accurately measure the color of an object as it is being manufactured. For instance, purchasers of paper frequently require the paper color to accurately match the color of previous purchases. Thus, paper manufacturers need to measure and control the color of paper to a previously determined value.

10 In the absence of fluorescence, the whiteness or color of an object is in general determined by the way the object absorbs and reflects light across the visible spectrum which when defined in terms of wavelength, is approximately 380 to 780 nanometers (nm). For example, white objects reflect light evenly across the spectrum while colored objects absorb some wavelengths (or color) and reflect others.

Color sensors typically illuminate the object and measure the intensity of the reflected light at each of a number of wavelengths. Each measured intensity of reflected light can be related to a previously measured 15 intensity of light which has been reflected from a white standard to provide a reflectance co-efficient at each wavelength. The set of reflectance co-efficients is often referred to as the color spectrum of the object.

Because paper naturally has a somewhat offwhite or straw color, fluorescent whitening agents (FWA) in the form of dyes are often added to the paper pulp to give the finished paper product a whiter appearance. Fluorescence is the phenomenon in which energy is absorbed over a range of wavelengths (the excitation 20 band) and then reemitted in a lower energy (longer wavelength) band referred to as the emission band. Fluorescent whitening agents typically absorb the violet and ultraviolet energies and remit this energy in the blue range to give the paper a whiter appearance.

The degree to which the object or paper illuminated by a particular source is made to appear bluer (or whiter) by the FWA depends upon the proportion of the energy emitted by the source in the violet and 25 ultraviolet regions as compared to the blue and other lower energy spectra, and also upon the effective concentration level of the fluorescent agent. Thus, if the excitation emission spectrum of the source changes, the amount of fluorescence and hence the color of the object will change. The emission spectra of various standard sources have been defined by the Commission Internationale de L' Eclairage (CIE) but attempts to build standard sources which exactly duplicate the defined spectra have been largely 30 unsuccessful. As a result, the emission spectra of the sources used in color sensors usually varies from sensor to sensor so that measured color spectra for a given fluorescent object also varies, although they may agree quite satisfactorily on non-fluorescent objects.

The effective FWA concentration level in an object has been measured using highly sophisticated techniques. In one such technique, a standard object having a known FWA concentration is first illuminated 35 by a source and the intensity of the light received from the object is measured. The object is then reilluminated after a filter which eliminates the light in the excitation band is placed over the source, and a second measurement is taken. The difference in these two measurements is directly related to the effective FWA concentration in the standard. The process is repeated for a sample of unknown FWA concentration and the two difference measurements are compared to determine the effective FWA concentration in the 40 sample.

This technique is typically very cumbersome since it involves the mechanical movement of a filter in front of the source and is therefore relatively slow. Consequently, it is difficult to rapidly measure the FWA concentration which makes the technique less practical for many on line applications such as measuring the FWA concentration in paper as it emerges from the paper making process. Also since the paper has moved 45 substantially between the two intervals of measurement, it cannot be assumed that the difference is solely a function of the FWA concentration. Any variation in a number of other attributes of the paper, such as opacity or whiteness, at the two points of measurement, could also affect the results.

In EP-A-181 155, a system is described for measuring the color of a sample material such as a sheet of paper. The system includes means for illuminating the sample and a sensor to measure light from the 50 sample. Sample backing means are provided to receive light transmitted by the sample and reflect this light back to the sample. The illuminating means include a light source producing light in the visible range, and a light source producing ultraviolet light. The intensities of light reflected by the sample at various frequencies are detected, giving a measurement of the color of the sample.

GB-A-822 447 describes a method and apparatus for the measurement and evaluation of fluorescent 55 substances, for example, "optical bleaches" in a sample such as paper or a textile material. Ultraviolet light (obtained by passing light from a suitable source through a U.V. filter) is directed at the sample containing the fluorescent substance. The reflected light is then passed through color filters placed one at a time in the path of the reflected light. The light transmitted through the color filter is detected by a photocell. From the

measurement made the fluorescent properties can be evaluated.

It is an object of the present invention to provide an improved method and apparatus of determining the fluorescent properties of a sample.

It is a further object of the present invention to provide an improved method and apparatus for determining the color spectrum for a sample which contains fluorescent agents if illuminated by a defined source.

According to the present invention there is provided a color sensor for detecting fluorescence of a fluorescent sample, the sensor comprising a first source of light positioned to illuminate the sample, and at least one light detector for detecting light reflected or emitted by the sample when illuminated by said first source, characterised in that the sensor additionally comprises a second source of light positioned to illuminate the sample, at least a portion of the light emitted by the second source being in the excitation band of the fluorescent sample, means for modulating the intensity of the second source independently of the first, and means for measuring the energy received from the sample in phase synchronism with the modulation of the second source.

According to the present invention there is further provided a method of determining the fluorescent property of a sample containing a fluorescent agent (FA), comprising the steps of: illuminating said sample with a first light source with light of a first spectrum; simultaneously to illuminating said sample with a second light source with light of a second spectrum, which second spectrum overlaps the excitation spectral range of said fluorescent agents to a greater degree than said first spectrum; simultaneously to modulating the intensity of said illumination by the second light source relative to the intensity of said illumination by the first light source; simultaneously to detecting the intensity emitted from said sample from both of said illuminations respectively, with a detector; and calculating the fluorescent property of said sample from the difference between the two intensity detections.

In an embodiment of the apparatus of the present invention a color sensor is provided which has two light sources for illuminating an object or sample. One light source, such as an ultraviolet source, emits light primarily in the excitation range of the fluorescent agent in the object. Data is taken in two interleaved phases. In phase 1, both sources are on and in phase 2, the source emitting energy primarily in the excitation range is turned off while the other source remains on.

In an embodiment of the apparatus of the present invention, a single measurement operation comprises accumulation of data in a relatively large number (≥ 10) of each of the two phases. This is advantageous since the accumulated data in each phase accurately represents the average product during the total measurement interval.

The sensor may be standardized by illuminating a standard of known fluorescent efficiency and known true reflectance (i.e., reflectance measured in the absence of fluorescent exciting radiation), to determine both the excitation energy of the ultraviolet source as well as the second source. Then, a sample of unknown FWA concentration may be illuminated by the sources and by comparing the data accumulated in each of the two phases during the measurement, the FWA concentration level can be determined. Furthermore, in another aspect of the present invention, the color spectrum of the sample if illuminated by an ideal CIE source or other defined source can be calculated.

The invention will be further described by way of example only with reference to the accompanying drawings in which:-

Figure 1 is a graph showing two measured color spectra of light received from a particular paper sample in which fluorescence is suppressed in one instance and fluorescence is present in the other;

Figure 2 is a graph illustrating the excitation and the emission spectra for a fluorescent whitening agent;

Figure 3 illustrates the respective spectra for two CIE defined sources;

Figure 4 is a schematic diagram of a color sensor in accordance with a preferred embodiment of the present invention;

Figure 5 illustrates two color spectra of a paper sample containing fluorescent whitening agent in which data has been taken 1) with the ultraviolet lamp of Figure 4 on, and 2) with the ultraviolet lamp off;

Figure 6 is a graph showing the difference spectrum of the data illustrated in Figure 5;

Figure 7 shows four color spectra for a paper sample containing a fluorescent whitening agent, two spectra of which are measured and two spectra of which are calculated;

Figure 8 is a schematic diagram of a driver circuit for the ultraviolet lamp of the color sensor of Figure 4;

Figure 9 is a timing diagram illustrating the two phases of the data acquisition; and

Figure 10 is an additional timing diagram for various waveforms of the driver circuit of Figure 8.

Figure 1 shows two color spectra, indicated at 10 and 12, which show the distribution of light received from an object (here a sample of white paper) as a function of wavelength when illuminated by different types of sources. The first color spectrum 10 depicts the intensity or brightness of the light received from

the sample for a range of wavelengths where fluorescence is absent or suppressed. The energy at each wavelength is expressed in terms of a reflectance co-efficient where 100 represents the reflection of a perfectly white object.

As shown in Figure 1, the color spectrum 10 drops off at the shorter wavelengths or higher energies which are at the blue-violet end of the spectrum. Because the yellow-red end of the spectrum is brighter, that is, more energy in the longer wavelengths is reflected by the sample than in the shorter wavelengths, the sample will appear beige or straw colored. In order to give the paper a whiter appearance, a fluorescent whitening agent (FWA) can be added to the paper during the paper making process to boost the blue wavelength energy received from the sample as represented by the color spectrum 12. Since the color spectrum 12 is relatively flat throughout the visible spectrum, the sample will have a white appearance.

As previously mentioned, the fluorescent whitening agents absorb light from the source which is in their excitation band and re-emit the light in a lower energy (longer wavelength) emission band. As shown in Figure 2, the excitation band for a typical fluorescent whitening agent is in the violet and ultraviolet range and the emission band is primarily in the blue range. Consequently, the degree to which a paper is made to appear bluer (and therefore whiter) by the FWA depends upon the balance of energy in the violet and ultraviolet regions (less than 430 nm) of the source as compared to the blue regions, as well as the effective FWA concentration.

The distribution of energy in terms of wavelength for two different CIE defined sources is illustrated in Figure 3. The distribution indicated at 18 is for the CIE standard source designated "C". The second distribution 20, for the CIE source "D65", indicates that the D65 source is brighter in the 300-400 nanometer range whereas the source C is brighter in the 400 to 500 nanometer range. Consequently, a paper sample with FWA is likely to appear bluer if illuminated by the source D65 as compared to the source C. However, the sources D65 and C as defined in Figure 3 like most sources are extremely difficult to accurately duplicate. Thus, even if two color sensors both utilize "D65" sources, the sensors are likely to yield somewhat different color spectra for a single sample containing FWA.

Referring now to Figure 4, a color sensor in accordance with a preferred embodiment of the present invention is indicated generally at 22. The color sensor 22 has a first source 24 of light which is used to illuminate the object to be tested, which in the illustrated embodiment is a sheet 26 of paper. The source 24 has a heat filter 28 to block infrared radiation and a color correcting filter 30 to redistribute the wavelengths at which the light is emitted.

The light source 24 is, in the illustrated embodiment, an incandescent tungsten lamp (modified by the color correcting filter so that its emission spectrum roughly approximates CIE illuminate "C") which therefore emits a significant amount of light in the ultraviolet or excitation range of fluorescent whitening agents. Accordingly, the paper 26 both reflects light from the source 24 and re-emits light absorbed from the source 24 in the emission range of the FWA.

The light reflected and emitted by the paper 26 is focused by a lens 34 through an aperture or slit 36 onto a folding mirror 40 of a wavelength dispersing assembly 42. The folding mirror 40 reflects the energy from the lens 34 onto a holographic grating 44 which disperses the energy by wavelength into a spectrum much like a rainbow and reflects the light back to the folding mirror 40A. The spectrum is reflected by the folding mirror 40A onto an array 46 of 32 diodes, each of which generates an electrical signal proportional to the total energy in the range of wavelengths which is directed to that particular diode.

The electrical output of each diode of the diode array 46 is sensed by a preamplifier and read by a computer 48 (as shown in the illustrated embodiment). The computer 48 includes a central processing unit or CPU 50, a memory 52, input/output (I/O) 54 and preamplifiers and multiplexers 56 for reading and processing the data from the diode array 46. The above-described elements 24-56 are well known to those skilled in the art and need not be described in greater detail.

In accordance with the present invention, the color sensor 22 has a second source 60 which emits light primarily in the excitation band of the fluorescent whitening agent. The second source 60 is an ultraviolet lamp in the illustrated embodiment, has a band pass filter 64, which transmits energy substantially in the excitation band of the FWA only. The second source 60 is rapidly turned on and off while the first source 24 remains on. As will be more fully discussed below, data is obtained while the second source 60 is on and also while the second source 60 is off. Differences in the two sets of data are used to compute the fluorescent whitening agent efficiency, or effective FWA concentration. Furthermore, a corrected color spectrum can be determined which would be obtained if the sample were illuminated by a defined source.

To calibrate the diodes of the diode array 46, the color sensor 22 has a plurality of standards 80 which are carried on a wheel 82. The standards include a very white sample which is moved in position by a stepper motor 84 to calibrate the sensor. Sensor calibration techniques are well known in the art.

After the sensor 22 has been calibrated, it is used to read a fluorescent standard sample with known

fluorescent properties in order to determine the excitation energy in the ultraviolet source 60 as well as in the incandescent source 24. Figure 5 shows an example of two color spectra which were obtained by the color sensor 22 when reading a sample with a high effective FWA concentration.

One diode of the diode array 46 is positioned to measure the energy of the light received from the sample at the 390 nanometer wavelength and another diode is positioned to measure the energy at the 700 nanometer wavelength, with the remaining 30 diodes being positioned to measure the energy at the wavelengths inbetween at 10 nanometer increments. The sensor produces the first spectrum A using both the UV source 60 and the incandescent source 24. In the illustrated embodiment, the outputs from the diode array 46 are read several times by the computer 48 to obtain an accumulated set of 32 data points during phase 1 in which both sources remain on.

In phase 2, the UV source 60 is turned off (while the incandescent source 24 remains on), and the output of the diode array 46 is again read several times to produce an accumulated phase 2 spectrum. As previously mentioned, the UV source 60 is cyclicly turned on and off, with the results of each cycle added to the previous accumulation. After a preset measurement interval, the computer stops data acquisition. An average intensity for each diode in phase 1 is then computed and stored in the computer as spectrum A for the fluorescent standard sample over the 390-700 nanometer range. Similarly, the intensity measurements from each diode of the diode array 46 while the UV source was off (phase 2) are averaged and stored by the computer producing the second spectrum B representing the energy of the light received from the standard sample when only the incandescent source 24 is on.

As shown in Figure 5, the light from the standard sample is brighter at the bluish end of the spectra when the UV source 60 is on. The scaled difference between the two color spectra A and B plotted in Figure 6 is stored in the computer. The increased brightness of the A spectrum is a result of the additional blue light emitted by the fluorescent whitening agent. The difference between the two color spectra A and B can be represented below as:

$$A_{\lambda} - B_{\lambda} = X_U \cdot F_{B_{\lambda}} \quad (1)$$

where X_U is the excitation energy in the UV source 60 and

$$F_{B_{\lambda}}$$

represents the efficiency of the FWA standard to produce fluorescent emission at wavelength λ when illuminated by a standard, fluorescence exciting source. Since the shape of the

$$F_{B_{\lambda}}$$

"spectrum" is fixed by the FWA dye emission curve, e.g., Fig. 2, it is sufficient to re-write equation (1) at a single wavelength:

$$A_{\lambda_p} - B_{\lambda_p} = X_U \cdot F_B \quad (1')$$

where we define

$$F_B \equiv F_{B_{\lambda_p}}$$

and the wavelength is chosen to be the peak wavelength, λ_p . We henceforth call F_B the 'fluorescent

coefficient' of the standard.

As previously mentioned, the fluorescent coefficient F_s of the fluorescent standard is known and equation (1) above may be rewritten as:

$$X_u = \frac{A_{\lambda_p} - B_{\lambda_p}}{F_s} \quad (2)$$

whereby the excitation energy X_u in the UV source 60 may be determined.

Equation (1) may also be rewritten as

$$X_u = \left\langle \frac{A_{\lambda} - B_{\lambda}}{F_s} \right\rangle_{\lambda_{\min} \rightarrow \lambda_{\max}} \quad (2')$$

where brackets denote an average of the quantity within over the wavelength range λ_{\min} to λ_{\max} . This average may reduce to measurement at a single wavelength λ_p as shown in equation (2) above, or may cover the entire emission band, for example.

An additional quantity which is important to be determined is the excitation energy X_s in the incandescent source 24 of the sensor 22. The quantity X_s can be determined in accordance with the following relationship:

$$(B_{\lambda_p} - C_{\lambda_p}) = X_s \cdot F_s \quad (3)$$

$$X_s = \frac{B_{\lambda_p} - C_{\lambda_p}}{F_s} \quad (4)$$

where the color spectrum C is the distribution of light which would be received from the standard sample if fluorescence was completely suppressed. In other words, the C spectrum shows the reflectance properties only of a sample, and is obtained for the standard sample using known laboratory techniques and equipment. The C spectrum for the standard sample is stored in the computer and is utilized after the B spectrum has been obtained to compute the excitation energy X_s in the incandescent source 24. In this manner, the source values X_u and X_s are calculated and stored as part of a standardization routine in which a standard sample is first read.

After the color sensor 22 has been standardized as described above, the color sensor 22 is ready to measure a sample of unknown fluorescent coefficient (FWA concentration). Figure 4 shows a sheet 26 of paper positioned to be read by the color sensor 22. The color sensor 22 may be used in a paper mill for example to measure the fluorescent properties and the color of paper as it is produced. Of course, the color sensor 22 is also suitable for color measurements of other objects and other fluorescent agents (FA).

To read the sample 26, the incandescent source 24 again remains on while the UV source 60 is cyclicly modulated on and off. The intensities measured by the color sensor 22 at the 32 wavelength increments yield two color spectra designated A' and B' as shown in Figure 7. As before, the color spectrum B' represents the measured relative intensities of the light received from the sample 26 when only the incandescent source 24 is on and the A' spectrum represents the measured relative intensities when the light from the UV source 60 is added to that of the incandescent source 24 to illuminate the sample 26.

Since the excitation energy X_u in the UV source 60 has been determined during the standardization routine, the fluorescent efficiency spectrum F_{λ} and the fluorescent coefficient, F' of the sample can be calculated by rearranging equations (1) and (2) as set forth below:

$$F'_{\lambda} = \frac{(A'_{\lambda} - B'_{\lambda})}{X_u} \quad (5)$$

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$$F' = \frac{(A'_{\lambda_p} - B'_{\lambda_p})}{X_u} \quad (5')$$

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Having determined the fluorescent efficiency of the sample 26, the color spectrum C' which would be obtained for the sample 26 if fluorescence was totally suppressed can be calculated by rearranging equation (4) as:

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$$C'_{\lambda} = B'_{\lambda} - (X_E \cdot F'_{\lambda}) \quad (6)$$

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where the excitation energy X_E in the incandescent source 24 is determined during the standardization step.

In the illustrated embodiment, the source excitation energies X_u and X_E and the fluorescent coefficient F' are calculated using the measured values of the color spectra A' and B' at the 450 nanometer wavelength because this has been found to be at or near the peak in the emission for most common FWA dyes.

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Having determined the fluorescent efficiency spectrum F'_{λ} and the suppressed fluorescence color spectrum C'_{λ} of the sample 26, a color spectrum D' which would be produced by the sample 26 if illuminated by an ideal CIE source can now be calculated where the excitation energy X_D in the CIE defined standard source is known. Such a spectrum is indicated at D' in Figure 7 and is calculated as set forth below:

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$$(D'_{\lambda} - C'_{\lambda}) = X_D \cdot F'_{\lambda} \quad (7)$$

or

$$D'_{\lambda} = C'_{\lambda} + (X_D \cdot F'_{\lambda}) \quad (8)$$

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It is seen that the spectrum D'_{λ} is fully corrected to a true standard defined source. The D'_{λ} spectrum can therefore be used for all subsequent calculations of color coordinates and whiteness or brightness for comparison purposes.

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Figure 8 shows a lamp driver circuit 100 for the UV source 60 of Figure 4. The driver circuit 100 includes an oscillator 102 which outputs a 35 kilohertz clock signal to a pulse generator 104. The computer 48 of Figure 4 outputs a 20 hertz "on/off" signal (depicted at (A) in Figure 9) to the pulse generator 104, to control the on/off modulation of the source 60 at a 20 hertz rate as depicted at (B) in Figure 9. Thus, the source 60 is on for 25 millisecond (ms) and off for 25 ms of each cycle.

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During the 25 ms on period, the source 60 is actually pulsed in the illustrated embodiment to increase lamp life. Accordingly, during the 25 ms on period of the source 60, the pulse generator 104 generates two drive signals, X and Y, which are coupled to the gates of two FET transistor switches 106 and 108, respectively. The drive signals X and Y are depicted at (A) and (B) in Figure 10 and each have a 30 microsecond period with a 3 microsecond pulse width which is set by an input 110 of the pulse generator 104. As shown in Figure 10, the drive signals X and Y are 180° out of phase, and thus the switches 106 and 108 are closed 180° out of phase.

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A transformer 112 having a primary coil 114 coupled to the switches 106 and 108 is energized by the alternate closing of the switches 106 and 108 by the drive signals X and Y, respectively. A secondary coil 116 of the transformer 112 produces an oscillating output voltage represented at (C) in Figure 10, which is applied across the UV source 60 through a ballast resistor 118. The oscillating voltage across the source causes the source 60 to pulse once every 15 microseconds as indicated at (D) in Figure 10. This rapid pulsing of the uv source 60 during the 25ms on period of the source 60 is represented by the vertical lines of the waveform at (B) in Figure 9. Because the preamplifiers 56 coupled to the outputs of the diode array

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46 have a time constant of approximately 2 1/2 milliseconds, the UV source 60 is effectively on constantly with respect to the array 46 during the 25 millisecond on period.

After the UV source 60 is turned on at the start of each 25 millisecond on period, the computer 48 waits approximately 10 milliseconds before reading the 32 diodes to allow sufficient time for the preamplifiers to respond to the new lighting condition. The 32 diodes are read 3 or 4 times at 5 millisecond intervals to obtain 3 or 4 sets of data for the 32 diodes while both the UV source 60 and the incandescent source 24 are on. During the 25 millisecond off period, the sequence is repeated, waiting 10 milliseconds before taking 3 or 4 sets of data while only the incandescent source 24 is on.

This cycle of taking data while the UV source 60 is off for 25ms and then while the UV source 60 is on for 25ms is repeated for approximately a second to define a data measurement interval or segment. Data from each diode while the UV source 60 was off is averaged over the whole data segment to produce the data points plotted in the B spectrum previously described. Similarly, the data for each diode taken while the UV source 60 was on is averaged over the whole data segment to produce the A spectrum. The rapid interleaving of data reads under the two lighting conditions reduces the effect of noise in the electronics and substantially eliminates the effect that slow drifts in electronics or optical efficiency or variation in the measured paper would otherwise have on the difference spectrum and hence the measurement of the fluorescent coefficient.

It is seen from the above that the present invention provides an improved color sensor which can calculate the fluorescent coefficient, related to the effective FWA concentration of a sample without the use of moving filters and the like. Moreover, the color sensor can generate a spectrum which is corrected to show the spectrum of the sample as it would be if illuminated by a defined source such as a CIE source, or other lighting sources in which the excitation energy is known. Consequently, the color of a particular sample can be predicted for a variety of lighting conditions.

It will, of course, be understood that modifications of the present invention, in its various aspects, will be apparent to those skilled in the art, some being apparent only after study, others being merely matters of routine electronic and mechanical design. For example, light detectors other than diodes may be utilized and other types of light sources may also be used. Other embodiments are also possible, with their specific designs dependent upon the particular application. As such, the scope of the invention should not be limited by the particular embodiment herein described, but should be defined only by the appended claims and equivalents thereof.

Claims

1. A color sensor (22) for detecting fluorescence of a fluorescent sample (26), the sensor comprising a first source (24) of light positioned to illuminate the sample, and at least one light detector (46) for detecting light reflected or emitted by the sample when illuminated by said first source, characterised in that the sensor additionally comprises a second source (60) of light positioned to illuminate the sample, at least a portion of the light emitted by the second source being in the excitation band of the fluorescent sample, means (104) for modulating the intensity of the second source independently of the first, and means for measuring the energy received from the sample in phase synchronism with the modulation of the second source.
2. A color sensor as claimed in claim 1, characterised in that said means (104) for modulating the intensity of the second source comprises means for modulating in a plurality of first and second phases wherein in each first phase the sample is illuminated by both the first and second sources and in each second phase any level of illumination of the sample by the second source differs from the level of illumination provided by the second source in the first phases.
3. A sensor as claimed in claim 2 wherein the means (104) for modulating the intensity of the second source (60) is adapted to switch the second source (60) on and off whilst the first source is switched on.
4. A sensor as claimed in claim 3 additionally comprising means for reading the energy detector (46) while both sources (24, 60) are on, and also while the first source is on and the second source is off.
5. A sensor as claimed in any one of claims 1 to 4 additionally comprising means (46, 48) for measuring at one or more wavelengths both the average intensity and the amplitude of the energy received from the sample (26).

6. An apparatus for determining the fluorescent property of a fluorescent sample (26), the apparatus comprising a sensor (22) as claimed in any one of claims 1 to 5 and further comprising means (48) for computing the fluorescent property based on the difference in detected levels of light resulting from said modulation.
- 5 7. Apparatus as claimed in claim 6 characterized in that the second light source (60) provides light with a second spectrum substantially overlapping the excitation spectral range of the fluorescent sample, and the first light source (24) provides light with a first spectrum which is less overlapping of the excitation spectral range of said fluorescent sample (26) than said second spectrum.
- 10 8. Apparatus as claimed in claim 7 wherein said second spectrum does not substantially overlap the wavelength regions outside the excitation spectral range of the fluorescent sample (26).
- 15 9. Apparatus as claimed in claim 6 which is for determining the color spectrum of said sample (26) wherein the first spectrum of said first light source is a defined spectrum, the second spectrum of said second light source is a defined spectrum, the detector (46) is capable of detecting energy levels at a plurality of spectral points within said first spectrum and said second spectrum, and the computing means is adapted to calculate, from the differences in the detected energy levels; at said spectral point resulting from said modulation, the color spectrum of said sample.
- 20 10. A method of determining the fluorescent property of a sample containing a fluorescent agent (FA), comprising the steps of:
 - illuminating said sample (26) with a first light source (24) with light of a first spectrum; simultaneously to
 - 25 illuminating said sample with a second light source (60) with light of a second spectrum, which second spectrum overlaps the excitation spectral range of said fluorescent agents to a greater degree than said first spectrum; simultaneously to
 - modulating the intensity of said illumination by the second light source relative to the intensity of said illumination by the first light source; simultaneously to
 - 30 detecting the intensity emitted from said sample from both of said illuminations, respectively, with a detector (46); and
 - calculating the fluorescent property of said sample from the difference between the two intensity detections.
- 35 11. A method as claimed in claim 10 for measuring the effective fluorescence of an object containing a fluorescent agent (FA), characterised in that said second source (60) emits a predetermined intensity of energy in the excitation range of the FA, and the effective fluorescence of the object is determined from the difference in the two intensity detections and said predetermined excitation intensity of the second source (60).
- 40 12. A method as claimed in claim 10 for determining the effective spectrum of a fluorescent sample as if illuminated by a defined source, further comprising the steps of:
 - sensing the intensity of the energy received from the sample at a plurality of wavelengths while the sample is illuminated with the first light source to provide a first color spectrum measurement;
 - 45 sensing the intensity of the energy received from the sample at said plurality of wavelengths while the sample is illuminated by the second source to provide a second color spectrum measurement;
 - determining the fluorescent coefficient of the sample from the difference in the two spectra measurements and the difference in the fluorescent excitation energy emitted by the first and second light source;
 - 50 determining the fluorescence suppressed spectrum of the sample from the known level of excitation energy from the first light source and the determined fluorescent coefficient and
 - determining the correct color spectrum of the sample as if it had been illuminated by a defined source from the determined fluorescent coefficient and the defined level of excitation energy of the defined source and the determined fluorescence suppressed spectrum.
- 55 13. A method as claimed in claim 10 for measuring the fluorescent efficiency spectrum F' in an unknown sample, comprising the steps of:
 - a) illuminating a standard sample of known fluorescent coefficient F_s with the first source (28) which

provides a first level X_s of excitation energy from a first source and detecting the level B_λ of energy received from the sample at one or more wavelengths;

b) illuminating the standard sample with the second source (60) which provides a level X_u of excitation energy and determining the intensity A_λ of energy received from the sample at said one or more wavelengths;

c) computing the level X_u of excitation energy from the second source as follows:

$$X_u = \left\langle \frac{A_\lambda - B_\lambda}{F_s} \right\rangle_{\lambda_{\min}}^{\lambda_{\max}}$$

d) illuminating the sample of unknown fluorescence with said first level of illumination and determining the intensity B'_λ of light received from the sample;

e) illuminating the unknown sample with both said first and second sources of illumination energy and determining the level A'_λ of energy received from the sample; and

computing the fluorescence efficiency spectrum F'_λ in the unknown sample as follows:

$$F'_\lambda = \frac{(A'_\lambda - B'_\lambda)}{X_u}$$

14. A method as claimed in claim 10 for computing the corrected spectrum of light energy received from a sample containing a fluorescent agent (FA) if illuminated by a defined source, comprising the steps of:

a) illuminating a standard sample of known fluorescent efficiency

$$F_{s\lambda}$$

with the first source (28) which provides a first level of illumination and detecting the spectrum B_λ of light energy received from the sample at a plurality of wavelengths λ ;

b) illuminating the standard sample with the second source (60) which provides a second level of energy and determining the spectrum A_λ of light received from the sample at said plurality of wavelengths;

c) computing the level X_u of increased excitation energy between the two levels as follows:

$$X_u = \left\langle \frac{A_\lambda - B_\lambda}{F_s} \right\rangle_{\lambda_{\min}}^{\lambda_{\max}}$$

d) computing the level X_s of excitation energy of said first level of illumination is follows:

$$X_s = \left\langle \frac{B_\lambda - C_\lambda}{F_s} \right\rangle_{\lambda_{\min}}^{\lambda_{\max}}$$

where the suppressed fluorescent spectrum C of the standard sample is known; and
 e) illuminating a sample of unknown fluorescence with said first level of energy and determining the spectrum B'_λ of light energy received from the sample at said plurality of wavelengths;
 f) illuminating the unknown sample with said second level of energy and determining the color spectrum A'_λ of light received from the sample at said plurality of wavelengths;
 5 g) computing the fluorescent efficiency F'_λ in the unknown sample as follows:

$$10 \quad F'_\lambda = \frac{(A'_\lambda - B'_\lambda)}{X_u}$$

h) computing the fluorescent suppressed color spectrum C'_λ of the unknown sample as follows:

$$15 \quad C'_\lambda = B'_\lambda - (X_s \cdot F'_\lambda);$$

20 i) computing the correct spectrum D'_λ of the unknown sample as if it had been illuminated by a defined source as follows:

$$25 \quad D'_\lambda = C'_\lambda + (X_D \cdot F'_\lambda)$$

where the level X_D of excitation energy of the defined source is known.

15. A method as claimed in claim 10 for sensing the fluorescent properties of a sample (26) containing fluorescent agents characterised in that the second light source (60) has a spectrum which overlaps the excitation spectral range of fluorescent agents to a greater degree than the spectrum of the first source (24), the energy received from said sample (26) resulting from said illuminations is detected in a sensor, and the fluorescent properties of the sample is calculated in electronic circuitry from said detected energy received in respect of said modulation.
- 35 16. A method as claimed in claim 15 wherein said second spectral range of the second light source (60) does not substantially overlap the wavelength regions outside said excitation spectral range of said fluorescent agents.
- 40 17. A method as claimed in claim 15 further comprising the step of determining the color spectrum of said sample (26), wherein said step of detecting further comprises:
 detecting in a sensor the energies received from said sample (26), resultant from said illuminations, at a plurality of spectral frequencies; and wherein said step of calculating further comprises:
 calculating from said detected energies the color spectrum of said sample.
- 45 18. A method as claimed in claim 15 wherein said first light source (24) is a tungsten lamp; and wherein said second light source (60) is an ultraviolet lamp, said first and second light sources being specifically designed for carrying out the method.
- 50 19. A method as claimed in any one of claims 10 to 18 wherein the modulation of the second light source (60) comprises switching said second light source on and off.

Patentansprüche

- 55 1. Farbensensor (22) zum Nachweisen von Fluoreszenz einer Fluoreszenzprobe (26), wobei der Sensor eine erste zur Beleuchtung der Probe in Stellung gebrachte Quelle (24) von Licht und zumindest einen Lichtdetektor (46) zum Nachweisen des bei einer Beleuchtung durch die genannte erste Quelle durch die Probe reflektierten oder abgestrahlten Lichtes umfaßt, dadurch gekennzeichnet, daß der Sensor

zusätzlich eine zweite zur Beleuchtung der Probe in Stellung gebrachte Quelle (60) von Licht, wobei zumindest ein Teil des durch die zweite Quelle abgestrahlten Lichtes im Anregungsband der Fluoreszenzprobe liegt, Mittel (104) zur Modulation der Intensität der zweiten Quelle unabhängig von der ersten und Mittel zur Messung der von der Probe her empfangenen Energie in Phasengleichheit mit der Modulation der zweiten Quelle, umfaßt.

2. Farbensensor nach Anspruch 1, dadurch gekennzeichnet, daß das genannte Mittel (104) zur Modulation der Intensität der zweiten Quelle Mittel zur Modulation in einer Vielzahl von ersten und zweiten Phasen umfaßt, worin in jeder ersten Phase die Probe sowohl durch die erste wie auch zweite Quelle beleuchtet wird und sich in jeder zweiten Phase jegliches Beleuchtungsniveau der Probe durch die zweite Quelle vom durch die zweite Quelle in den ersten Phasen gelieferten Beleuchtungsniveau unterscheidet.

3. Sensor nach Anspruch 2, worin das Mittel (104) zur Modulation der Intensität der zweiten Quelle (60) so beschaffen ist, daß es die zweite Quelle (60) ein- und ausschaltet, während die erste Quelle eingeschaltet ist.

4. Sensor nach Anspruch 3, der zusätzlich Mittel zum Ablesen des Energiedetektors (46), während beide Quellen (24, 60) an sind, und auch während die erste Quelle an und die zweite Quelle aus ist, umfaßt.

5. Sensor nach einem beliebigen der einzelnen Ansprüche 1 bis 4, der zusätzlich Mittel (46, 48) zur Messung bei einer oder mehreren Wellenlängen sowohl der mittleren Intensität wie auch der Amplitude der von der Probe (26) her empfangenen Energie umfaßt.

6. Vorrichtung zur Bestimmung der Fluoreszenzeigenschaft einer Fluoreszenzprobe (26), wobei die Vorrichtung einen Sensor (22) nach einem beliebigen der einzelnen Ansprüche 1 bis 5 umfaßt und dabei ferner Mittel (48) zur Berechnung der Fluoreszenzeigenschaft auf der Grundlage des Unterschieds zwischen nachgewiesenen Niveaus des sich aus der genannten Modulation ergebenden Lichtes umfaßt.

7. Vorrichtung nach Anspruch 6, dadurch gekennzeichnet, daß die zweite Lichtquelle (60) Licht mit einem zweiten mit dem spektralen Anregungsbereich der Fluoreszenzprobe im wesentlichen überlappenden Spektrum liefert und die erste Lichtquelle (24) Licht mit einem ersten Spektrum liefert, das eine geringere Überlappung mit dem spektralen Anregungsbereich der genannten Fluoreszenzprobe (26) als das genannte zweite Spektrum aufweist.

8. Vorrichtung nach Anspruch 7, worin das genannte zweite Spektrum mit den Wellenlängenbereichen außerhalb des spektralen Anregungsbereichs der genannten Fluoreszenzprobe (26) nicht wesentlich überlappt.

9. Vorrichtung nach Anspruch 6 zur Bestimmung des Farbenspektrums der genannten Probe (26), worin es sich beim ersten Spektrum der genannten ersten Lichtquelle um ein definiertes Spektrum handelt, es sich beim zweiten Spektrum der genannten zweiten Lichtquelle um ein definiertes Spektrum handelt, der Detektor (46) den Nachweis von Energieniveaus bei einer Vielzahl von Spektralpunkten innerhalb des genannten ersten Spektrums und des genannten zweiten Spektrums erbringen kann und das Berechnungsmittel beschaffen ist, aus den Unterschieden zwischen den nachgewiesenen Energieniveaus am genannten, sich aus der genannten Modulation ergebenden Spektralpunkt das Farbenspektrum der genannten Probe zu berechnen.

10. Verfahren zur Bestimmung der Fluoreszenzeigenschaft einer ein Fluoreszenzmittel (FA) enthaltende Probe, welches folgende Schritte umfaßt:

eine Beleuchtung der genannten Probe (26) mit einer ersten Lichtquelle (24) mit Licht eines ersten Spektrums; gleichzeitig zur

Beleuchtung der genannten Probe mit einer zweiten Lichtquelle (60) mit Licht eines zweiten Spektrums, welches zweite Spektrum mit dem spektralen Anregungsbereich der genannten Fluoreszenzmittel in stärkerem Maße als das genannte erste Spektrum überlappt; gleichzeitig zur

Modulation der Intensität der genannten Beleuchtung durch die zweite Lichtquelle bezüglich der Intensität der genannten Beleuchtung durch die erste Lichtquelle; gleichzeitig zum

Nachweis der von der genannten Probe jeweils von beiden der genannten Beleuchtungen her abgestrahlten Intensität mit einem Detektor (46) und eine

Berechnung der Fluoreszenzeigenschaft der genannten Probe aus dem Unterschied zwischen den zwei Intensitätsnachweisen.

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11. Verfahren nach Anspruch 10 zur Messung der effektiven Fluoreszenz eines ein Fluoreszenzmittel (FA) enthaltenden Gegenstandes, dadurch gekennzeichnet, daß die genannte zweite Quelle (60) eine vorbestimmte Intensität von Energie im Anregungsbereich des FA abstrahlt und die effektive Fluoreszenz vom Gegenstand aus dem Unterschied zwischen den beiden Intensitätsnachweisen und der

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12. Verfahren nach Anspruch 10 zum Bestimmen des effektiven Spektrums einer Fluoreszenzprobe, als ob durch eine definierte Quelle beleuchtet, welches weiter folgende Schritte umfaßt:

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eine Aufnahme der Intensität der von der Probe her bei einer Vielzahl von Wellenlängen empfangenen Energie, während die Probe mit der ersten Lichtquelle beleuchtet wird, um eine erste Farbenspektrummessung zu liefern; eine

Aufnahme der Intensität der von der Probe her bei der genannten Vielzahl von Wellenlängen empfangenen Energie, während die Probe durch die zweite Quelle beleuchtet wird, um eine zweite Farbenspektrummessung zu liefern; eine

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Bestimmung des Fluoreszenzkoeffizienten der Probe aus dem Unterschied zwischen den beiden Spektrenmessungen und dem Unterschied zwischen der Fluoreszenzanregungsenergie, die von der ersten und zweiten Lichtquelle abgestrahlt wird; eine

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Bestimmung des Spektrums bei unterdrückter Fluoreszenz der Probe aus dem bekannten Niveau der Anregungsenergie von der ersten Lichtquelle und dem bestimmten Fluoreszenzkoeffizienten; und eine

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Bestimmung des korrekten Farbenspektrums der Probe, als ob sie durch eine definierte Quelle beleuchtet worden wäre, aus dem bestimmten Fluoreszenzkoeffizienten und dem bestimmten Niveau der Anregungsenergie der definierten Quelle und dem bestimmten Spektrum bei unterdrückter Fluoreszenz.

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13. Verfahren nach Anspruch 10 zur Messung der Fluoreszenzausbeute F' in einer unbekannten Probe, welches folgende Schritte umfaßt:

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a) eine Beleuchtung einer Standardprobe mit bekanntem Fluoreszenzkoeffizienten F_s mit der ersten Quelle (28), welche ein erstes Niveau X_s der Anregungsenergie aus einer ersten Quelle liefert, und einen Nachweis des Niveaus B_λ der von der Probe her bei einer oder mehreren Wellenlängen empfangenen Energie; eine

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b) Beleuchtung der Standardprobe mit der zweiten Quelle (60), welche ein Niveau X_u der Anregungsenergie liefert, und eine Bestimmung der Intensität A_λ der von der Probe her bei den genannten ein oder mehreren Wellenlängen empfangenen Energie; eine

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c) Berechnung des Niveaus X_u der Anregungsenergie von der zweiten Quelle her auf die folgende Weise:

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$$X_u = \left\langle \frac{A_\lambda - B_\lambda}{F_s} \right\rangle \frac{\lambda_{\max}}{\lambda_{\min}} ;$$

60

eine

d) Beleuchtung der Probe mit unbekannter Fluoreszenz mit genanntem ersten Beleuchtungsniveau und eine Bestimmung der Intensität B'_λ des von der Probe her empfangenen Lichtes; eine

e) Beleuchtung der unbekannten Probe mit beiden genannten ersten und zweiten Quellen von Beleuchtungsenergie und eine Bestimmung des Niveaus A'_λ der von der Probe her empfangenen Energie; und eine

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Berechnung des Spektrums der Fluoreszenzausbeute F'_λ in der unbekannten Probe auf die folgende Weise:

$$F'_{\lambda} = \frac{(A'_{\lambda} - B'_{\lambda})}{X_u}$$

14. Verfahren nach Anspruch 10 zur Berechnung des korrigierten Spektrums der von einer ein Fluoreszenzmittel (FA) enthaltenden Probe her empfangenen Lichtenergie wenn durch eine definierte Quelle beleuchtet, welches folgende Schritte umfaßt:

- a) eine Beleuchtung einer Standardprobe mit bekannter Fluoreszenzausbeute $F_{s\lambda}$ mit der ersten Quelle (28), welche ein erstes Beleuchtungsniveau liefert, und einen Nachweis des Spektrums B_{λ} der von der Probe her bei einer Vielzahl von Wellenlängen λ empfangenen Energie; eine
- b) Beleuchtung der Standardprobe mit der zweiten Quelle (60), welche ein zweites Energieniveau liefert, und eine Bestimmung des Spektrums A_{λ} des von der Probe her bei der genannten Vielzahl von Wellenlängen empfangenen Lichtes; eine
- c) Berechnung des Niveaus X_u der zugenommenen Anregungsenergie zwischen den beiden Niveaus auf die folgende Weise:

$$X_u = \left\langle \frac{A_{\lambda} - B_{\lambda}}{F_s} \right\rangle_{\lambda_{\min}}^{\lambda_{\max}} ;$$

eine

- d) Berechnung des Niveaus X_s der Anregungsenergie des genannten ersten Beleuchtungsniveaus auf die folgende Weise:

$$X_s = \left\langle \frac{B_{\lambda} - C_{\lambda}}{F_s} \right\rangle_{\lambda_{\min}}^{\lambda_{\max}}$$

wobei das Spektrum bei unterdrückter Fluoreszenz C der Standardprobe bekannt ist; und eine

- e) Beleuchtung einer Probe mit unbekannter Fluoreszenz mit genanntem ersten Energieniveau und eine Bestimmung des Spektrums B'_{λ} der von der Probe her bei der genannten Vielzahl von Wellenlängen empfangenen Lichtenergie; eine
- f) Beleuchtung der unbekannten Probe mit dem genannten zweiten Energieniveau und eine Bestimmung des Farbenspektrums A'_{λ} des von der Probe her bei der genannten Vielzahl von Wellenlängen empfangenen Lichtes; eine
- g) Berechnung der Fluoreszenzausbeute F'_{λ} in der unbekannten Probe auf die folgende Weise:

$$F'_{\lambda} = \frac{(A'_{\lambda} - B'_{\lambda})}{X_u} ;$$

eine

- h) Berechnung des Farbenspektrums bei unterdrückter Fluoreszenz C'_{λ} der unbekannten Probe auf die folgende Weise:

$$C'_\lambda = B'_\lambda - (X_s * F'_\lambda);$$

eine

i) Berechnung des korrekten Spektrums D'_λ der unbekannten Probe, als ob sie durch eine definierte Quelle beleuchtet worden wäre, auf die folgende Weise:

$$D'_\lambda = C'_\lambda + (X_D * F'_\lambda)$$

wobei das Niveau X_D der Anregungsenergie der definierten Quelle bekannt ist.

15. Verfahren nach Anspruch 10 zur Aufnahme der Fluoreszenzeigenschaften einer Fluoreszenzmittel enthaltenden Probe (26), dadurch gekennzeichnet, daß die zweite Lichtquelle (60) ein mit dem spektralen Anregungsbereich der Fluoreszenzmittel in stärkerem Maße als das Spektrum der ersten Quelle (24) überlappendes Spektrum aufweist, wobei die sich aus den genannten Beleuchtungen ergebende, von der genannten Probe (26) her empfangene Energie in einem Sensor nachgewiesen wird, und die Fluoreszenzeigenschaften der Probe aus der genannten nachgewiesenen, bezüglich der genannten Modulation empfangenen Energie in elektronischen Schaltkreisen berechnet werden.

16. Verfahren nach Anspruch 15, worin der genannte zweite Spektralbereich der zweiten Lichtquelle (60) mit den Wellenlängenbereichen außerhalb des genannten spektralen Anregungsbereichs der genannten Fluoreszenzmittel nicht wesentlich überlappt.

17. Verfahren nach Anspruch 15, weiter umfassend den Schritt einer Bestimmung des Farbenspektrums der genannten Probe (26), worin der genannte Schritt des Nachweisens weiter umfaßt:

einen Nachweis in einem Sensor der von der genannten Probe (26) her auf die genannten Beleuchtungen hin empfangenen Energiegehalte bei einer Vielzahl spektraler Frequenzen, und worin der genannte Schritt des Berechnens weiter umfaßt:

eine Berechnung des Farbenspektrums der genannten Probe aus den genannten nachgewiesenen Energiegehalten.

18. Verfahren nach Anspruch 15, worin es sich bei der genannten ersten Lichtquelle (24) um eine Wolframlampe handelt, und worin es sich bei der genannten zweiten Lichtquelle (60) um eine ultraviolette Lampe handelt, wobei die genannten erste und zweite Lichtquellen zur Durchführung des Verfahrens speziell konstruiert sind.

19. Verfahren nach einem beliebigen der einzelnen Ansprüche 10 bis 18, worin die Modulation der zweiten Lichtquelle (60) das Ein- und Ausschalten der genannten zweiten Lichtquelle umfaßt.

Revendications

1. Capteur de couleurs (22) destiné à détecter la fluorescence d'un échantillon fluorescent (26), le capteur comprenant une première source lumineuse (24) placée de façon à éclairer l'échantillon, et au moins un détecteur de lumière (46) destiné à détecter la lumière réfléchie ou émise par l'échantillon lorsqu'il est éclairé par ladite première source, caractérisé en ce que le capteur comprend en outre une seconde source lumineuse (60) placée de façon à éclairer l'échantillon, une partie au moins de la lumière émise par la seconde source se trouvant dans la bande d'excitation de l'échantillon fluorescent, un moyen (104) de modulation de l'intensité de la seconde source indépendamment de la première, et un moyen de mesure de l'énergie reçue en provenance de l'échantillon en synchronisme de phase avec la modulation de la seconde source.

2. Capteur de couleur selon la revendication 1, caractérisé en ce que ledit moyen (104) de modulation de l'intensité de la seconde source comprend un moyen de modulation en une pluralité de premières et de secondes phases, dans lequel, au cours de chaque première phase, l'échantillon est éclairé à la fois par la première et la seconde source, et au cours de chaque deuxième phase tout niveau d'éclairage de l'échantillon par la seconde source diffère du niveau d'éclairage fourni par la seconde source dans les premières phases.

3. Capteur selon la revendication 2 dans lequel le moyen (104) de modulation de l'intensité de la seconde

source (60) est adapté pour allumer et éteindre la seconde source (60) lorsque la première source est allumée.

4. Capteur selon la revendication 3 comprenant en outre un moyen de lecture du détecteur d'énergie (46) lorsque les deux sources (24, 60) sont allumées et également lorsque la première source est allumée et la seconde source éteinte.
5. Capteur selon l'une quelconque des revendications 1 à 4 comprenant en outre des moyens (46, 48) pour la mesure à une ou plusieurs longueurs d'onde de l'intensité moyenne et de l'amplitude de l'énergie reçue en provenance de l'échantillon (26).
6. Appareil pour déterminer la propriété fluorescente d'un échantillon fluorescent (26), cet appareil comprenant un capteur (22) selon l'une quelconque des revendications 1 à 5 et comprenant en outre un moyen (48) de calcul de la propriété fluorescente basé sur la différence entre des niveaux lumineux détectés résultant de ladite modulation.
7. Appareil selon la revendication 6 caractérisé en ce que la seconde source lumineuse (60) fournit une lumière dans un second spectre qui chevauche sensiblement la bande spectrale d'excitation de l'échantillon fluorescent, et la première source lumineuse (24) fournit une lumière dans un premier spectre qui chevauche moins la bande spectrale d'excitation dudit échantillon fluorescent (26) que ledit second spectre.
8. Appareil selon la revendication 7 dans lequel ledit second spectre ne chevauche pas sensiblement les domaines de longueur d'onde extérieurs à la bande spectrale d'excitation de l'échantillon fluorescent (26).
9. Appareil selon la revendication 6 qui est destiné à déterminer le spectre de couleurs dudit échantillon (26) dans lequel le premier spectre de ladite première source lumineuse est un spectre défini, le second spectre de ladite seconde source lumineuse est un spectre défini, le détecteur (46) est capable de détecter des niveaux d'énergie à une pluralité de points spectraux à l'intérieur dudit premier spectre et dudit second spectre, et le moyen de calcul est adapté pour calculer, à partir des différences entre les niveaux d'énergie détectés, audit point spectral résultant de ladite modulation, le spectre de couleurs dudit échantillon.
10. Méthode pour la détermination de la propriété fluorescente d'un échantillon contenant un agent fluorescent (FA), comprenant les étapes suivantes:
 - éclairage dudit échantillon (26) par une première source lumineuse (24) ayant une lumière d'un premier spectre; en même temps que
 - éclairage dudit échantillon par une seconde source lumineuse (60) ayant une lumière d'un second spectre, lequel second spectre chevauche la bande spectrale d'excitation desdits agents fluorescents de façon plus importante que ledit premier spectre; en même temps que
 - modulation de l'intensité dudit éclairage par la seconde source lumineuse par rapport à l'intensité dudit éclairage par la première source lumineuse; en même temps que
 - détection de l'intensité émise par ledit échantillon à partir desdits deux éclairages, respectivement, à l'aide d'un détecteur (46); et
 - calcul de la propriété fluorescente dudit échantillon à partir de la différence entre les deux détections d'intensité.
11. Méthode selon la revendication 10 pour la mesure de la fluorescence effective d'un objet contenant un agent fluorescent (FA), caractérisée en ce que ladite seconde source (60) émet une intensité prédéterminée d'énergie dans la bande d'excitation de l'agent fluorescent et que la fluorescence effective de l'objet est déterminée à partir de la différence entre les deux détections d'intensité et ladite intensité d'excitation prédéterminée de la seconde source (60).
12. Méthode selon la revendication 10 pour la détermination du spectre effectif d'un échantillon fluorescent comme s'il était éclairé par une source définie, comprenant en outre les étapes suivantes:
 - détection de l'intensité de l'énergie reçue en provenance de l'échantillon à une pluralité de

longueurs d'onde lorsque l'échantillon est éclairé par la première source lumineuse pour fournir une première mesure de spectre de couleurs,

détection de l'intensité de l'énergie reçue en provenance de l'échantillon à la dite pluralité de longueurs d'onde lorsque l'échantillon est éclairé par la seconde source de façon à obtenir une deuxième mesure de spectre de couleurs;

détermination du coefficient de fluorescence de l'échantillon à partir de la différence entre les deux mesures de spectres et de la différence d'énergie d'excitation fluorescente émise par la première et la seconde source lumineuse;

détermination du spectre de fluorescence supprimée de l'échantillon à partir du niveau connu d'énergie d'excitation en provenance de la première source lumineuse et du coefficient de fluorescence déterminé, et

détermination du spectre de couleurs correct de l'échantillon comme si celui-ci avait été éclairé par une source définie, à partir du coefficient de fluorescence déterminé et du niveau défini d'énergie d'excitation de la source définie et du spectre de fluorescence supprimée déterminé.

13. Méthode selon la revendication 10 pour la mesure du spectre de rendement fluorescent F' d'un échantillon inconnu, qui comprend les étapes suivantes:

a) éclairage d'un échantillon standard ayant un coefficient de fluorescence connu F_s par la première source (28) qui fournit un premier niveau X_s d'énergie d'excitation à partir d'une première source, et détection du niveau B_λ d'énergie reçue en provenance de l'échantillon à une ou plusieurs longueurs d'onde ;

b) éclairage de l'échantillon standard par la seconde source (60) qui fournit un niveau X_u d'énergie d'excitation, et détermination de l'intensité A_λ de l'énergie reçue en provenance de l'échantillon à ladite une ou plus longueur(s) d'onde;

c) calcul du niveau X_u d'énergie d'excitation en provenance de la seconde source comme suit:

$$X_u = \left\langle \frac{A_\lambda - B_\lambda}{F_s} \right\rangle_{\lambda \min}^{\lambda \max}$$

d) éclairage de l'échantillon de fluorescence inconnue par ledit premier niveau d'éclairage, et détermination de l'intensité lumineuse B'_λ reçue en provenance de l'échantillon;

e) éclairage de l'échantillon inconnu par à la fois lesdites première et seconde sources d'énergie d'éclairage, et détermination du niveau A'_λ d'énergie reçue en provenance de l'échantillon; et calcul du spectre de rendement de fluorescence F'_λ de l'échantillon inconnu comme suit:

$$F'_\lambda = \frac{(A'_\lambda - B'_\lambda)}{X_u}$$

14. Méthode selon la revendication 10 pour le calcul du spectre corrigé d'énergie lumineuse reçue en provenance d'un échantillon contenant un agent fluorescent (FA) si celui-ci est éclairé par une source définie, qui comprend les étapes suivantes:

a) éclairage d'un échantillon standard ayant un rendement de fluorescence connu $F_{s\lambda}$ par la première source (28) qui fournit un premier niveau d'éclairage, et détection du spectre B_λ d'énergie lumineuse reçue en provenance de l'échantillon à une pluralité de longueurs d'onde λ ;

b) éclairage de l'échantillon standard par la seconde source (60) qui fournit un second niveau d'énergie, et détermination du spectre lumineux A_λ reçu en provenance de l'échantillon à ladite pluralité de longueurs d'onde ;

c) calcul du niveau X_u d'énergie d'excitation accrue entre les deux niveaux comme suit:

$$X_u = \frac{A_\lambda - B_\lambda}{F_s} \quad \begin{matrix} \lambda \text{ max} \\ \lambda \text{ min} \end{matrix}$$

d) calcul du niveau X_s d'énergie d'excitation dudit premier niveau d'éclairage comme suit:

$$X_s = \frac{B_\lambda - C_\lambda}{F_s} \quad \begin{matrix} \lambda \text{ max} \\ \lambda \text{ min} \end{matrix}$$

dans lequel le spectre C de fluorescence supprimée de l'échantillon standard est connu; et

e) éclairage d'un échantillon de fluorescence inconnue par ledit premier niveau d'énergie et détermination du spectre B'_λ d'énergie lumineuse reçue en provenance de l'échantillon à ladite pluralité de longueurs d'ondes;

f) éclairage de l'échantillon inconnu par ledit second niveau d'énergie et détermination du spectre de couleurs A'_λ de lumière reçue en provenance de l'échantillon à ladite pluralité de longueurs d'onde;

g) calcul du rendement de fluorescence F'_λ de l'échantillon inconnu comme suit:

$$F'_\lambda = \frac{(A'_\lambda - B'_\lambda)}{X_u}$$

h) calcul du spectre de couleurs à fluorescence supprimée C'_λ de l'échantillon inconnu comme suit:

$$C'_\lambda = B'_\lambda - (X_s \cdot F'_\lambda);$$

i) calcul du spectre correct D'_λ de l'échantillon inconnu comme si celui-ci avait été éclairé par une source définie comme suit:

$$D'_\lambda = C'_\lambda + (X_D \cdot F'_\lambda)$$

dans lequel le niveau X_D d'énergie d'excitation de la source définie est connu.

15. Méthode selon la revendication 10 pour détecter les propriétés fluorescentes d'un échantillon (26) contenant des agents fluorescents, caractérisée en ce que la seconde source lumineuse (60) a un spectre qui chevauche la bande spectrale d'excitation des agents fluorescents de manière plus importante que le spectre de la première source (24), l'énergie reçue en provenance dudit échantillon (26) en conséquence desdits éclairages est détectée par un capteur, et les propriétés fluorescentes de l'échantillon sont calculées dans des éléments de circuit électronique à partir de ladite énergie détectée reçue relativement à ladite modulation.

16. Méthode selon la revendication 15 dans laquelle ladite seconde bande spectrale de la seconde source lumineuse (60) ne chevauche pas sensiblement les domaines de longueur d'onde situés en dehors de ladite bande spectrale d'excitation desdits agents fluorescents.

17. Méthode selon la revendication 15 comprenant en outre l'étape de détermination du spectre de couleurs dudit échantillon (26), dans laquelle ladite étape de détection comprend en outre:

la détection par un capteur des énergies reçues en provenance dudit échantillon (26) en consé-

quence desdits éclairages, à une pluralité de fréquences spectrales: et dans laquelle ladite étape de calcul comprend en outre:
le calcul du spectre de couleurs dudit échantillon, à partir desdites énergies détectées.

- 5 18. Méthode selon la revendication 15 dans laquelle ladite première source lumineuse (24) est une lampe au tungstène; et dans laquelle ladite seconde source lumineuse (60) est une lampe à rayons ultraviolets, lesdites première et seconde sources lumineuses étant spécialement conçues pour l'application de cette méthode.
- 10 19. Méthode selon l'une quelconque des revendications 10 à 18 dans laquelle la modulation de la seconde source lumineuse (60) comprend l'allumage et l'extinction de ladite seconde source lumineuse.

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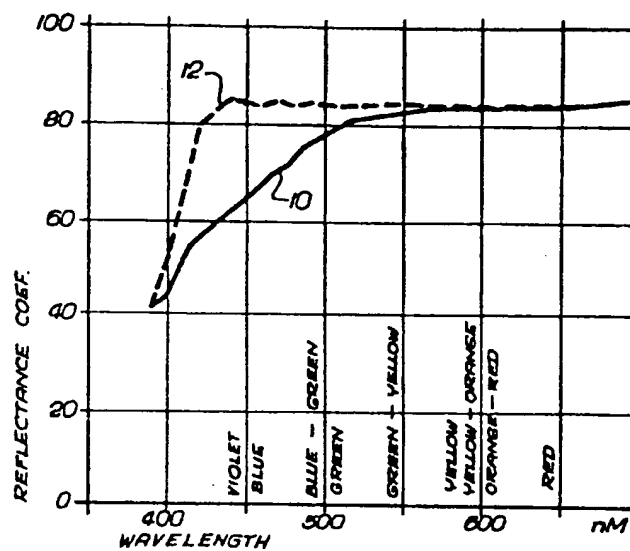
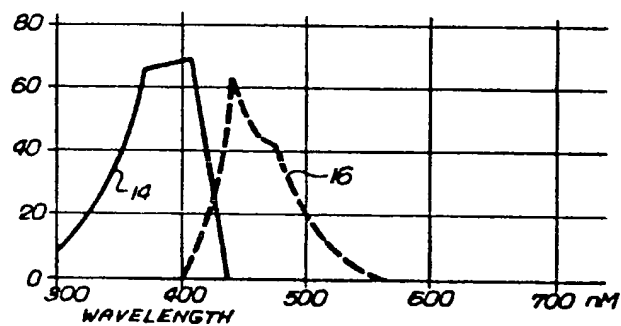
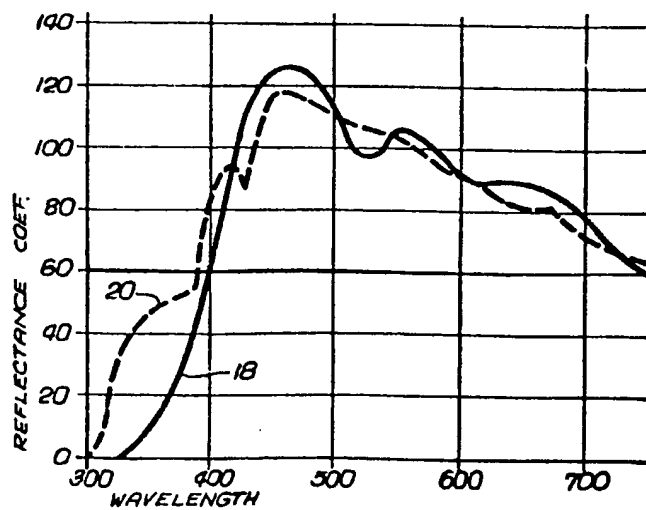
Fig. 1*Fig. 2**Fig. 3*

Fig. 4

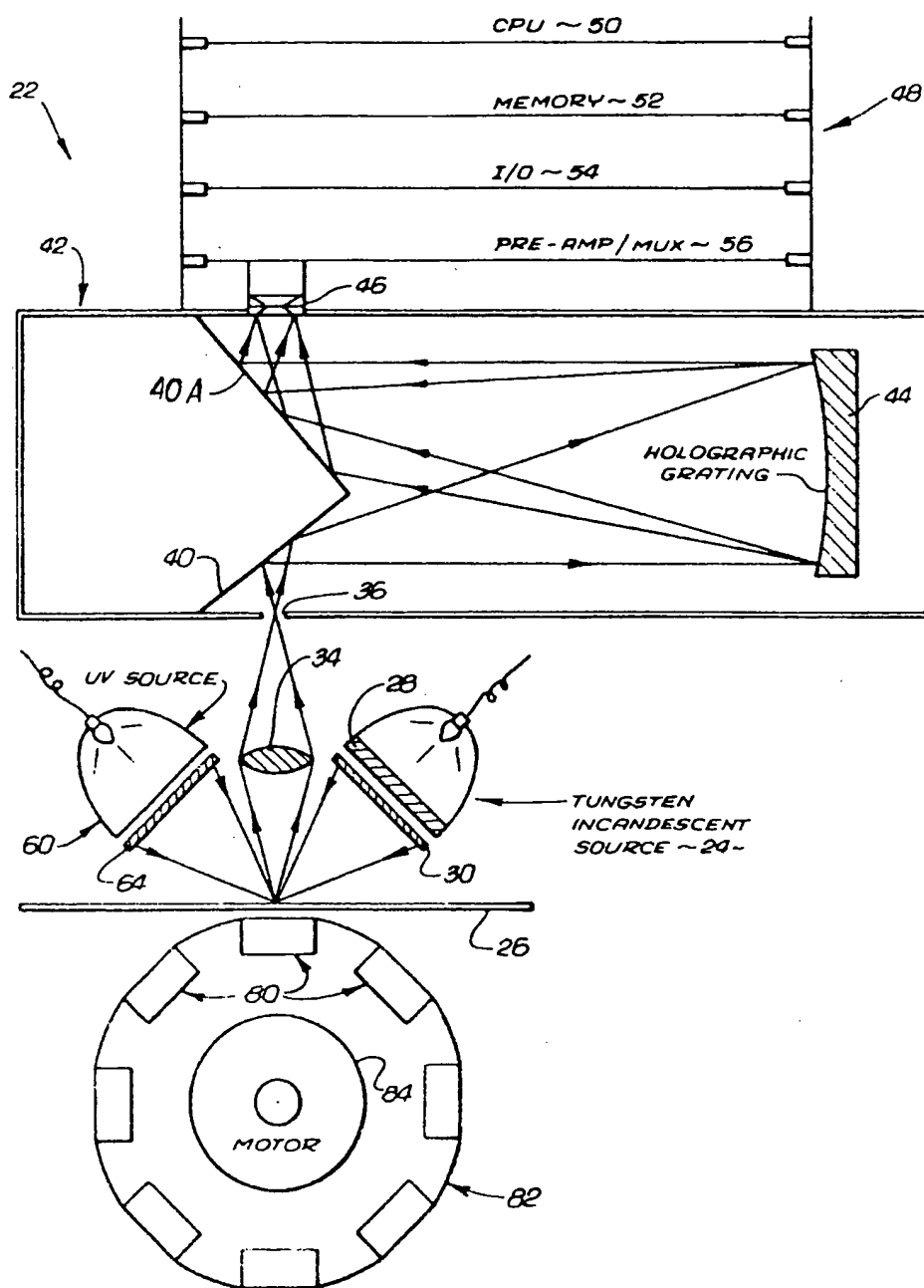


FIG. 5

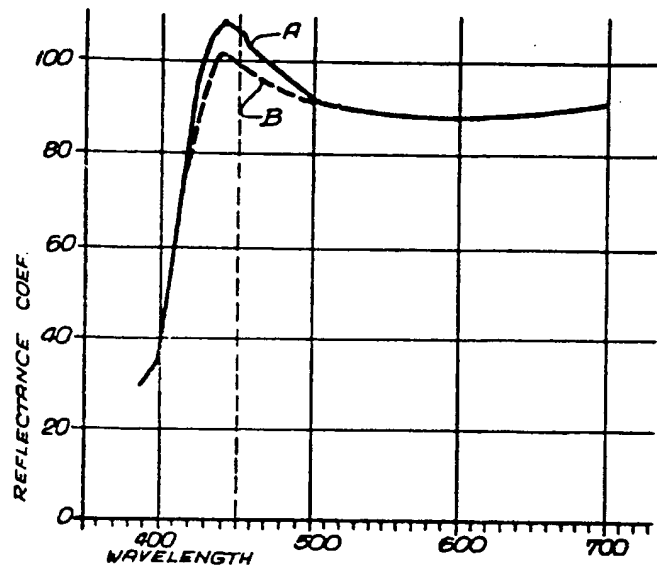


FIG. 6

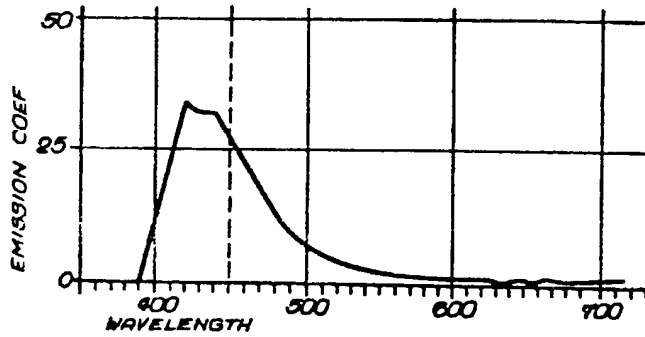


FIG. 7

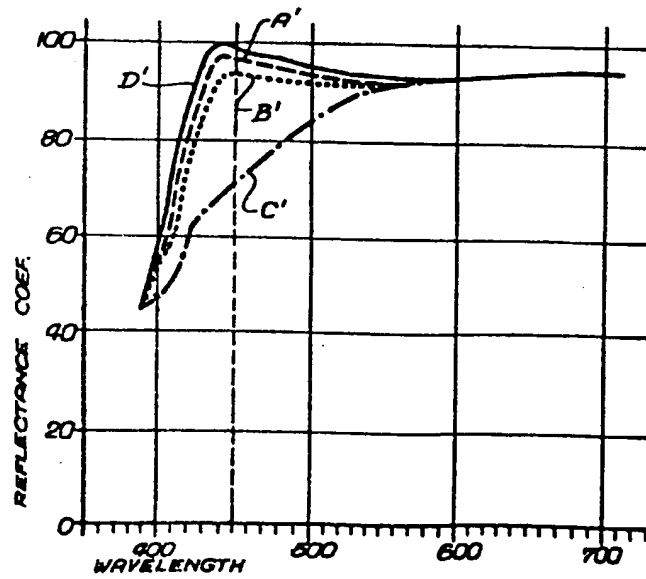
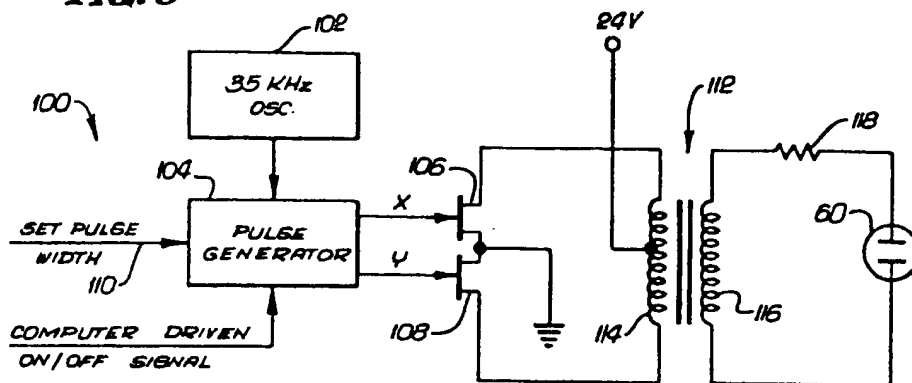
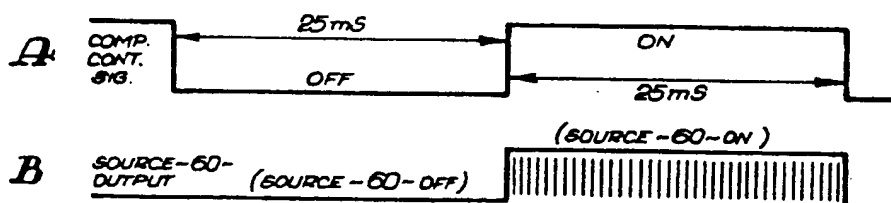
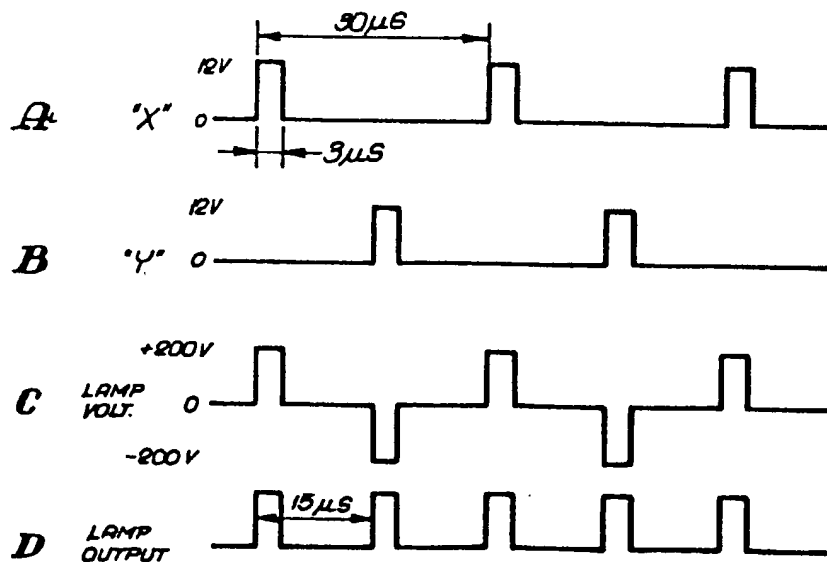


Fig. 8**Fig. 9****Fig. 10**

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